

WHAT IS CLAIMED IS:

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1. A method for identifying essential and non-essential genes in a genome of a cell grown in non-selective conditions, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;

amplifying a target region from said non-selected sub-population of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and

assessing for the presence or absence of said first and second extension product, whereby the presence of the first and second extension products is indicative of a non-essential gene, whereas the presence of the first extension product and the absence of the second extension product is indicative of an essential gene.

2. A method according to claim 1, wherein mutagenizing is performed with a transposable element.

3. A method according to claim 2, wherein said target DNA comprises a gene encoding a protein.

4. A method for functional analysis of a target region in a sequence of interest, said method comprising:

mutagenizing said target region by insertion of a sequence tag to provide a population of DNA molecules containing a sequence tag insertion in at least 90% of nucleotide positions in said target region;

introducing said population of mutagenized DNA molecules
into host cells that express said sequence of interest;

subjecting a first aliquot of said host cells to at least one selective condition and a second aliquot to a non-selective condition to provide at least one selected and one non-selected aliquot;

amplifying said target region from said at least one selected and one non-selected aliquots, using a first primer hybridizing to said sequence tag and a second primer hybridizing to a known endpoint, said endpoint being characterized as an arbitrary unique sequence in said target DNA, to provide amplified DNA; and

resolving by gel electrophoresis said amplified DNA from said at least one selected and one non-selected aliquots into individual bands differing by size to identify the position of individual sequence tag insertions within said target region,

whereby differences between the presence or intensity of bands between said at least one selected and one non-selected aliquots are indicative that said sequence tag insertion causes a difference in response to said selective condition employed with said at least one selected aliquot resulting in the functional analysis of said target region.

5. A method according to claim 4, wherein mutagenizing comprises the steps of:

combining DNA comprising said target region with retroviral integrase and a first set of complementary oligonucleotide primers, said primers comprising (a) a recognition sequence for said retroviral integrase and (b) a sequence tag, wherein said retroviral integrase mediates the insertion of said first set of complementary oligonucleotide primers to provide a population of mutagenized DNA molecules.

6. A method according to claim 4, wherein mutagenizing comprises the steps of:

combining DNA comprising said target region with retroviral integrase and a first set of complementary oligonucleotide primers, said primers comprising (a) a recognition sequence for said retroviral integrase and (b) a recognition site for a type IIs restriction endonuclease, wherein said retroviral integrase mediates the insertion of said first set of complementary oligonucleotide primers to provide a population of mutagenized DNA molecules

cutting said population of mutagenized DNA molecules with said type IIs restriction endonuclease to provide cut DNA; and

ligating to said cut DNA a second set of complementary oligonucleotide primers comprising a sequence tag.

7. A method according to claim 5, wherein said sequence of interest comprises a gene encoding a protein.

8. A method according to claim 4, wherein said selective condition is growth of cells in media lacking a nutrient that is an intermediate in a metabolic pathway.

9. A method according to claim 8, wherein said population of mutagenized DNA molecules are cloned into a filamentous bacteriophage vector with regulatory sequences for expression of said sequence of interest.

10. A method according to claim 5, wherein said sequence of interest comprises a regulatory gene.

11. A method according to claim 10, wherein said selective condition is growth in media containing a cytotoxic agent, and said regulatory gene controls expression of a gene conferring resistance to said cytotoxic agent.

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12. A method according to ~~one of claims 4 to 11~~, whereby the absence of a band under said selective condition and its presence under non-selective conditions is indicative of a target region which is essential under said selective condition.

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13. A method according to ~~one of claims 1, 12~~, wherein said genome is a haploid genome.

14. A method according to claim 13, wherein said haploid genome is a bacterial genome.

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15. A method for identifying essential genes in a genome of a cell grown in non-selective conditions, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;

amplifying a target region from said non-selected subpopulation of cells, using a first primer which hybridizes to a known end of said target region, and a second primer which hybridizes to said oligonucleotide sequence, said first and second primers constituting a primer pair capable of giving rise to an amplification of an extension product when said oligonucleotide sequence is inserted into said target region; and

assessing for the presence or absence of said first and second extension product, whereby the presence thereof is indicative of a non-essential gene, whereas the absence thereof is indicative of an essential gene.

16. A method for identifying essential genes in a genome of a cell comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under selective or non-selective conditions to provide a selected or non-selected sub-population of cells;

amplifying a target region from said sub-population of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and

assessing for the presence or absence of said first and second extension product, whereby the presence of the first and second extension products is indicative of a non-essential gene, whereas the presence of the first extension product and the absence of the second extension product is indicative of an essential gene.

17. A method according to claim 16, wherein said genome is a haploid genome.

18. A method according to claim 16 to 18, wherein insertion mutagenesis is carried out with a transposable element.

19. A method according to ~~one of claims 1-18~~, wherein said amplification is carried out by the polymerase chain reaction.

20. A method for identifying a therapeutic target in a genome of a cell grown in non-selective conditions, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;

amplifying a target region from said non-selected subpopulation of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a

second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and

assessing for the presence or absence of said first and second extension product, whereby the presence of the first extension product and the absence of the second extension product is indicative of an essential gene and hence of an identification of a therapeutic target in said cell.

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Year	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	